

KARYOTYPES AND CASE STUDIES OF
17 DOWN'S SYNDROME INDIVIDUALS

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17 DOWN'S SYNDROME INDIVIDUALS

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An abstract of a Thesis by
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The problem. Sixteen patients suspected of having mosaic Down's syndrome and one suspected of having a translocation Down's syndrome were studied by chromosome analysis at Woodward State Hospital, Woodward, Iowa during June and July of 1973.

Procedure. A cytogenetic analysis was made of chromosomes prepared from leucocyte cultures from peripheral blood. At least 30-50 cells were counted and karyotypes made for each patient.

Findings. The cell counts and karyotypic studies verified the presumption that 16 patients had mosaic Down's syndrome and that one patient had translocation Down's syndrome. The chromosome counts showing 46 chromosomes in the mosaic individuals were normal chromosome complements. The individual shown to have a translocation type of Down's syndrome appeared to be carrying an unbalanced translocation involving chromosome 14 and an extra chromosome number 21.

Conclusion. The observed frequency of 1.15% of translocation Down's syndrome at Woodward is lower than the 3-4% reported by other investigators. The 18.4% frequency for mosaic type Down's at Woodward is much higher than the 1-2% reported by other authors. The mean age found for those individuals with mosaicism in this study is higher than those with trisomy 21, although it is not statistically significant. No significant difference in the prevalence of mosaics among patients born to young or old mothers was evident.

Recommendations. A follow-up study to determine if the mortality rate of mosaic type Down's syndrome individuals is significantly lower than those with trisomy 21 is recommended.

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INTRODUCTION AND REVIEW OF LITERATURE

There has been considerable research conducted in regard to the causes of Down's syndrome since this anomaly was originally termed mongoloid idiocy or mongolism by Langdon Down in 1866 (Hamerton, 1971a). Langdon Down (1866) described these individuals as "strumous cretins" and referred to them as "mongols." By the late nineteenth century numerous reports describing patients with Down's syndrome had appeared (Smith, 1971). An ethnographic study showed that these patients did not have physical features characteristic of the Mongolian race. Allen et al. (1961) suggested that since the original terms of mongoloid idiot and mongol carried a racial implication, they should be dropped. Following this suggestion, the term Down's syndrome has become the accepted term for this disorder (Hamerton, 1971a). Down's syndrome will be used throughout this paper to refer to the disorder being studied.

The clinical features of Down's syndrome have been described many times (Oster, 1953; Penrose, 1961; Beckman et al., 1962; Valentine, 1969). Valentine (1969) listed some of the features of Down's syndrome as follows: I. Q. varying between 20 and 60, short stature, tendency to be overweight, hypotonia, shortness of limbs, square hands with short, stubby fingers, short broad feet with a poorly developed arch, a wide gap between the big toe and next toe

is often noticeable, brachycephalic, a short and thick neck, eyes are set closer together than normal because of the under-development of the skull, low set ears, epicanthal fold, persistent obstruction and infection of upper respiratory tract possibly due to small size of post-nasal space and airway, enlarged tongue which protrudes from the mouth and often becomes fissured, narrow acetabular and iliac angle and congenital heart disease is quite common. Acute leukemia is at least three times more common in a person with Down's syndrome than in normal individuals while Penrose (1961) reported 20 times greater incidence than the general population.

The normal human chromosome number of 46 was identified with certainty by Tjio and Levan (1956). After this number was established, Down's syndrome was the first disorder in which a chromosomal abnormality was found. Lejeune et al. (1959) reported that patients with Down's syndrome had 47 chromosomes with the extra chromosome consistently in the smallest acrocentric or group G chromosomes. The small acrocentric chromosome involved is traditionally called 21 (Denver Conference, 1960). Chromosome number 21 is the smallest chromosome of the G group and as a consequence it should be named 22 since it would be smaller than the chromosome which is actually now being called 22. However, the situation is that the chromosome number 21 has been associated with Down's syndrome and so the convention, which

is a departure from the strict rules of nomenclature, points out that Down's syndrome is also trisomy 21 rather than trisomy 22 as it would be designated if the strict observance of the rules were met (Hamerton, 1971b). It is only possible in very few cases when using conventional staining methods to distinguish pair number 21 from the other G chromosome pair number 22 on morphological grounds. If fluorescent or Giemsa banding techniques were used it is possible to routinely make a distinction between chromosome pair 21 and chromosome pair 22. Down's syndrome involving three number 21 chromosomes is referred to as trisomy 21 and is designated by the symbols 47, XY, or XX, +21) referring to a male or female with 47 chromosomes including an additional number 21 chromosome (Paris Conference, 1971). The standardized nomenclature set up at the Paris Conference (1971) will be the nomenclature used throughout this paper in designating the various types of Down's syndrome.

The majority of individuals with Down's syndrome have regular trisomy 21, and a small percentage have either translocation Down's syndrome or chromosome mosaicism with more than a single cell population. Mikkelsen (1967) found the incidence of trisomy 21 to be 89%, of translocation in Down's syndrome to be 9% and the frequency of chromosome mosaicism among patients with typical Down's syndrome to be 2% from a total of 100 patients. Chitham and MacIvan (1965) reported 92.38% trisomy 21, 4.75% translocations and 2.85%

mosaics from a statistical survey of 105 cases of Down's syndrome. Ziska (1970) in a cytogenetic study of 112 cases of Down's syndrome reported 91.07% cases of trisomy 21, 7.14% translocations and 1.78% mosaic type Down's syndrome. Hayashi (1963) in a karyotypic analysis of 79 cases of Down's syndrome found 93.67% with 21-trisomy, 3.67% translocations and 2.41% mosaics. Richards et al. (1965) reported 95.11% trisomy 21 Down's syndrome, 2.22% translocations and 2.67% mosaics out of 225 patients. Higurashi et al. (1969) reported 93.45% trisomy 21 Down's syndrome, 4.36% translocations and 2.18% mosaics from 321 cases in Japan. Edgren et al. (1966) in a cytogenetic study of 73 patients with Down's syndrome reported 97.26% of the normal trisomy 21 and 2.74% mosaics. There were no translocations which appeared in this particular study.

The work of these individuals seems to verify the fact that the most common type of Down's syndrome is trisomy 21 and that the least common types are due to translocations and chromosome mosaicism. These studies also point out that the least common type of Down's syndrome is due to chromosome mosaicism. An average of these seven cytogenetic studies involving a total of 1015 individuals of Down's syndrome shows that 93.3% were due to trisomy 21, 4.43% to translocation and 2.26% to chromosome mosaicism.

Waardenburg (1932) correctly surmised that Down's syndrome is caused by an extra chromosome. Mittwoch (1952)

believed that certain human abnormalities might be caused by chromosomal aberrations and examined the chromosome complements of cells from the testis of an individual with Down's syndrome but failed to detect the aneuploidy which we now know exists. Lejeune et al. (1959) made the important discovery that trisomy 21 is the developmental consequence of aneuploidy. It has been indicated by Robinson (1973) that the first meiotic division is the most common time for the non-disjunction to occur. Robinson examined the chromosomes of 15 families including father, mother, and the child affected with Down's syndrome using the fluorescence staining technique. The child's number 21 chromosomes were identified in terms of the phenotype of the parental chromosomes. Her results showed that in five cases where positive identification could be made (four standard trisomies and one translocation trisomy) that the error occurred at the first meiotic division in the mother. Other cases were either impossible to detect or were listed as possibly occurring in the first meiotic division or the second in either the mother or the father.

Penrose (1933) analyzed 150 sibships, each containing at least one Down's syndrome individual, with respect to the relative etiological importance of paternal age and maternal age. The results indicated that paternal age is not a significant factor, while maternal age is to be regarded as very important. Shuttleworth (1909), Jenkins (1933), Penrose

(1934, 1961), Valentine (1969) and Smith (1971) also pointed out that the risk of trisomy 21 occurring in the progeny increases with the age of the mother.

The first translocation in man was described by Turpin et al. (1959) and with time, it has become increasingly obvious that translocations are one of the most common types of chromosome abnormalities found in man (Jacobs et al., 1970). One of the first cases of translocation Down's syndrome was described by Polani et al. (1960). A girl with Down's syndrome, in contrast with reported chromosome findings for Down's syndrome, had only 46 chromosomes in her bone-marrow cells including four small acrocentric chromosomes characteristic of normal females. There were only five, instead of six, of the longer acrocentric chromosomes in the D group (13-15) but an extra chromosome was present that was not distinguishable from a C group chromosome. A reciprocal translocation between a chromosome in the D group and 21 was suggested by Polani et al. (1960) as the origin of the anomaly. Breaks occur near the centromeric region of a chromosome from the D group as well as from one of the G group (21-22) and lead to a reciprocal exchange. Two chromosomes are produced, a large translocation chromosome with the genetic material of the long arms of both chromosomes and a microchromosome consisting of the short arms and satellites of both chromosomes. The large translocation chromosome can be transmitted through several

generations, while the small translocation chromosome is lost during cell division (Mikkelsen, 1971). Translocations between acrocentric chromosomes are called Robertsonian or "centric fusion" translocations (Robertson, 1916). Penrose et al. (1960) were the first to describe a family where a balanced 13-15/21 [45, XX or XY, -G, +t (Gq Dq)] translocation was demonstrated in normal relatives of the affected individual.

Chromosome pairs 13, 14, and 15 have been shown to be distinguishable by autoradiographic analysis of their DNA replication patterns (Schmid, 1963; Yunis et al., 1964; German, 1964; Giannelli, 1965; Gey, 1966). Chromosomally normal cells labeled late in their DNA synthetic period have been found to show a pair of chromosomes (Number 13) with heavy label over the middle and distal portions of the long arms, a pair of chromosomes (Number 14) with heavy label over the centromere and short arms, and a pair of chromosomes (Number 15) with very light or no label. Autoradiographic studies by Hecht et al. (1968) suggest that the chromosome involved in a D/G translocation is usually Number 14, rarely Number 15 and never Number 13. The results from 20 patients with Down's syndrome carrying a 13-15/21 translocation indicated that chromosome 14 was involved in eighteen cases and chromosome 15 in two cases.

Whereas there is an increased risk of Down's syndrome due to trisomy 21 with an increase in maternal age,

transmission of a translocation chromosome is expected to occur independently of maternal age. Thus, the greatest number of these cases will occur in the younger maternal age group coincident with the greatest number of total births (Peterson and Luzzatti, 1965).

Translocations can be inherited if one of the parents carries the translocation (Wright et al., 1967). The parent will have one chromosome 21 and one free chromosome 14 and a large translocation chromosome generally composed of part of chromosome 14 and part of chromosome 21. All of the critical genetic material is present, therefore the gametes produced by this parent can be expected to include: (1) normal 14 and 21, producing a normal zygote; (2) chromosome 14 alone, producing a zygote monosomic for chromosome 21; (3) translocation chromosome 14-21 and normal 21 (an unbalanced translocation), producing a Down's syndrome zygote; (4) translocation chromosome alone (a balanced translocation), resulting in a phenotypically normal individual with 45 chromosomes as in the parent; (5) chromosome 21 alone, producing a zygote monosomic for chromosome 14; (6) translocation chromosome 14-21 and normal 14 producing a zygote trisomic for 14 (McKusick, 1964).

The alternative type of translocation besides an inherited translocation is a sporadic translocation in which the parents have normal chromosome complements and it is assumed that the translocation is a new mutational event

(Wright et al., 1967). Wright et al. (1967) also reported that in the maternal age group less than 30 years old about 75% of the translocations were sporadic in the affected offspring and 25% were inherited. In the maternal age over 30 years old 85% of the translocations were sporadic and 15% were inherited. The carrier parent can generally be detected by cytogenetic studies and should be sought in all cases of translocation Down's syndrome. There is a high risk of the translocation carrier producing another child with Down's syndrome or progeny who could be a carrier of the translocation. Family studies of 13-15/21 translocation Down's syndrome have shown that either the father or the mother may be the carrier. Large families produced by a carrier parent may contain several individuals with Down's syndrome. Theoretically, if neither parent is considered to be a carrier, the risk of having another child with Down's syndrome is less than if either parent was a carrier (Eber and Goodman, 1966).

Another kind of translocation is one involving an attachment of chromosomes 21 and 22 in the G group. Theoretically a carrier of a true 21/21 translocation would produce only offspring with Down's syndrome. Some of these carrier parents do produce normal children indicating that they carry a 21/22 translocation. Meiosis would produce gametes similar to those produced from segregation of 13-15/21 translocations with respect to proportions of nonviable

gametes, balanced translocations, unbalanced translocations, and normal gametes (Eber and Goodman, 1966).

An isochromosome is formed when a centromere divides transversely rather than longitudinally during anaphase. The resulting isochromosome 21 has the genetic material of long arms of two 21 chromosomes or short arms of two 21 chromosomes (Valentine, 1969). After fertilization, the zygote will be abnormal by being either trisomic or monosomic for a portion of the 21 chromosome. A zygote monosomic for an autosome is nonviable. The condition of the zygote which is either trisomic or monosomic for a number 21 chromosome is exactly the same when the abnormal chromosome is a 21/21 translocation. All viable children will be affected with Down's syndrome (Mikkelsen, 1971).

Mosaic Down's syndrome was first reported by Clark et al. (1961) in a female infant with some features of mongolism and two cell types, one normal and the other trisomic for a small acrocentric chromosome. This is evidently the most common kind of mosaic Down's syndrome but triple stem cell mosaicism, normal, trisomic, and tetrasomic for a small acrocentric chromosome and mosaicism associated with structural change, usually centric fusion of two small acrocentrics, have been reported (Richards, 1969). Richards (1969) reported that among 51 mosaics there was one instance of triple stem mosaicism and three of mosaicism with structural change, all being translocations or isochromosomes involving two

small acrocentrics. There have been no reports to my knowledge of 14/21 translocation Down's syndrome/normal mosaics.

Mosaicism is due to mitotic nondisjunction of chromosomes during early embryonic stages. If both daughter chromosomes of an autosomal chromosome migrate to the same daughter cell a monosomic and a trisomic cell will result. Since the monosomic cell is not viable and since the trisomic cell will undergo further mitosis, the resulting mosaic individual will have normal and trisomic cell lines. This individual would be referred to as a 46/47 mosaic. Mitotic nondisjunction is rarer than meiotic non-disjunction and therefore mosaicism is rarer than whole body trisomy 21 (Zellweger, 1968). Anaphase lag involving a number 21 chromosome at one of the first mitotic divisions of a trisomic zygote will also result in a 46/47 mosaic (Mikkelsen, 1971).

Jacobson (1967) pointed out that three clinical variants of mosaicism in young mothers have been described: the general mosaic where the mixture of cells causes an intermediate phenotypic expression between the normal individual and a patient showing marked signs of Down's syndrome; the regional mosaic where expression is determined by the organs affected; the gametic mosaic where the only abnormal tissues is in the gonad and a 50:50 (normal:trisomic) reproductive outcome is observed. Mosaicism with a large population of trisomy 21 cells will result in an individual who is generally phenotypically indistinguishable from trisomy 21 or

translocation Down's syndrome (Zellweger, 1968).

If mosaic cell lines are found in one of the parents, the risk of affected offspring is impossible to predict accurately (Mikkelsen, 1971).

Using standard procedures for preparing chromosome spreads of leucocytes cultured from human peripheral blood such as those of Moorhead et al. (1960) and staining techniques which produce bands on the chromosomes such as those of Seabright (1971), it has become increasingly easier to karyotype and identify specific chromosomes involved in translocations, trisomies, and other chromosomal aberrations.

The individuals in this study are presumptive mosaic Down's syndrome individuals which were ascertained in a previous study (Pieper, 1973). Using the information available at Woodward State Hospital, case studies of each of these individuals were examined. These findings were used in conjunction with cytogenetic analysis in order to complete the diagnosis on each individual and also to try and distinguish most accurately whether these individuals are truly mosaic for their chromosome complement.

MATERIALS AND METHODS

In this investigation 17 residents of Woodward State Hospital, clinically diagnosed to have Down's syndrome, were studied. Sixteen of these were suspected to be mosaic for Down's syndrome and one patient was suspected of being a

translocation Down's syndrome individual (Pieper, 1973). Thirty to fifty cells of each individual were counted to determine the number of chromosomes present. Cells showing good chromosome spreads with relaxed chromosome arms, good morphology and no excessive over-lapping were selected for photographs. Karyotypes were made from these photographs to determine: (1) which chromosomes were involved in the patient suspected of being a translocation Down's syndrome individual; (2) if the individuals who are presumptive mosaics have a normal 46 chromosome cell line or if this 46 chromosome count is due to a translocation; (3) the relative proportion of normal and trisomic cells in each demonstrated mosaic.

The procedures followed for culturing, harvesting, and fixing the leucocytes were modified from Moorhead et al. (1960). The culture medium was 85 ml Minimal Essential Medium (Eagle) with Hanks' salts and L - Glutamate plus 15 ml fetal calf serum (GIBCO). One liter of the culture medium was prepared, placed into ten separate sterile bottles and frozen for future use.

Before collecting blood, the culture medium was thawed and one milliliter of penicillin (10,000 units/ml) / streptomycin (10,000mcg/ml) and one milliliter of phytohemagglutinin were added to each 100 ml of culture medium. Using 30 ml tissue culture flasks (Falcon Plastics), 8 ml of culture medium was placed in each flask. The flasks containing

the medium were stored at 37°C for about a 24 hour period.

Ten milliliters of peripheral venous blood were drawn by the medical technician at Woodward State Hospital from each of the residents being examined. The blood was drawn aseptically using a 10 ml plastic syringe and transferred to a sterile culture tube containing 150 units of sodium heparin. Blood and sodium heparin were mixed gently and the tube was allowed to stand for three hours. At the end of this time, the plasma layer containing the needed lymphocytes had separated from the red blood cells.

Using a sterile Pasteur pipette for each blood sample and drawing the lymphocytes from the buffy layer, two milliliter samples of plasma were added to each of the culture flasks containing the medium. The culture flasks were then laid flat, swirled gently and placed in an incubator at 37°C for 72 hours before harvesting. Two hours before harvesting the cells, 0.5 ml of reconstituted Colcemid (10 mcg/ml) (GIBCO) was added to each of the culture flasks.

The cells were harvested by transferring the contents of the culture flasks into clean centrifuge tubes which had been washed thoroughly, rinsed 10 times in tap water and rinsed three to five times in distilled water. The cell suspensions were centrifuged for 12 minutes at 800 rpm and all but 0.5 ml of the supernatant fluid was removed. The packed cells were then resuspended in the remaining supernatant fluid. Three milliliters of 0.075M potassium chloride

at 37°C was added slowly to each of the tubes. The suspension was incubated at 37°C for seven minutes and centrifuged for five minutes. The supernatant liquid was then aspirated off.

In fixing the cells, three to four milliliters of freshly prepared fixative consisting of one part glacial acetic acid and three parts absolute methanol (Carnoy's Solution) were added slowly down the side of the centrifuge tube without disturbing the button of cells. The cells were then allowed to soak in the fixative for 30 minutes. The cells were resuspended and centrifuged for five minutes. The supernatant liquid was then aspirated off. The cells were resuspended in three or four milliliters of fresh fixative, allowed to stand for five minutes and centrifuged for five minutes more. This last procedure was then repeated one more time to completely disperse the clumps of cells. The supernatant liquid was aspirated off, 0.25 - 0.5 ml of fresh fixative was added to the button of cells and the cells were resuspended.

Cells were fixed to microscope slides which had been acid cleaned, rinsed thoroughly with distilled water and chilled in a beaker of distilled water. To prepare a slide, excess water was shaken off of it and three or four drops of the hazy cell suspension were added to the slide. The slide was then brought into contact momentarily with a flame to ignite the fixative over the surface. As soon as the

fixative burned off, the slide was blown on gently and then allowed to air dry completely. Ten slides were made from each resident being tested.

The procedures for staining for banding were modified from (Seabright, 1971). A phosphate buffered saline (PBS) was prepared as follows:

NaCl (8000 mg/liter); KCl (200 mg/liter);

Na_2HPO_4 (1150 mg/liter); KH_2PO_4 (200 mg/liter).

This solution was adjusted to a pH of 7.2 ± 0.1 with 5N NaOH, measured out in 10 bottles of 100 ml each and frozen. This PBS was used at room temperature.

Stock bottles of 0.25% concentration of trypsin (GIBCO) were used to prepare 100 ml of 0.1% stock solution. Forty milliliters of the 0.25% trypsin were added to 60 ml of the PBS Solution to prepare a 0.1% stock solution. A working solution of 0.001% trypsin (GIBCO) was prepared in PBS buffer solution and was used at room temperature.

One milliliter of Gurr's Giemsa R66 stain was added to each 50 ml of Gurr's buffer (6.8pH) to prepare the final staining solution. The slides were placed in 0.001% trypsin solution for one minute. The slides were then immediately rinsed in 70% methanol for two minutes, in 100% methanol for another two minutes and finally rinsed in distilled water. The slides were then placed in the Gurr's Giemsa stain for

90 minutes, rinsed briefly in distilled water and air dried.

The cells were observed and the chromosomes counted at 1250X using oil immersion bright field optics of a Zeiss Photomicroscope II. Metaphase cells for these counts were selected on the basis that the arms of the chromosomes were relaxed, free from undue curling and showed little or no over-lapping (Pieper, 1973). Other criteria used included selection of those metaphase cells that showed the sharpest detail in chromosome structure and banding whenever it was possible to distinguish distinct bands. Selected metaphase spreads and configurations were photographed with 35mm Panatomic X film in order to verify questionable counts as well as for use in karyotyping. Prints were made on Kodabromide F-5 paper. Criteria used in selecting cells for photographing were the same as those used in selecting cells for counting.

Case studies were conducted on each of the 17 patients involved in this research. Information collected, as available, included birth date, age, sex, pregnancy history, delivery, history of miscarriages, age of parents at birth of patient, consanguinity, abnormalities in other family members, order of birth, unusual illnesses and physical features. This information was obtained from records compiled by personnel at Woodward State Hospital.

RESULTS

In the present study 17 patients were clinically diagnosed as having Down's syndrome. Sixteen of these were presumptive mosaics and one was suspected of having Down's syndrome of the translocation type. Cell counts and karyotypic studies were done on each of the patients. The cell counts and karyotypic studies verified the above presumptions. The 46 chromosome counts observed in mosaic individuals were normal chromosome complements and did not represent a translocation cell line. In the individual suspected of being a translocation type Down's syndrome there appeared to be an unbalanced translocation involving chromosome number 14 and an extra chromosome number 21. Cell counts for each of the individuals studied are given in Table 1 and are also included in the text. Table 2 shows the patient's sex, birthdate, age and I. Q., and parental ages at birth of the patient.

The mean ages of the 16 mosaics studied were found to be 25.875 years. The mean of the original 87 patients studied by Pieper (1973) excluding the 16 mosaics is 22.253 years. This difference of 3.622 is not significant using a standard t-test.

Case reports on each individual are given below based on the information available at Woodward State Hospital.

TABLE 1. Cell counts for patients studied at Woodward State Hospital.

Index Number	Number of cells counted			Total
	45 chromosomes	46 chromosomes	47 chromosomes	
101	0	11	20	31
102	0	16	25	41
103	1	12	23	36
104	2	10	18	30
105	0	20	10	30
106	1	18	19	38
107	3	38	0	41
108	2	18	14	34
109	1	16	13	30
110	1	22	19	42
111	0	18	18	36
112	0	15	20	35
113	0	19	29	48
114	0	14	26	40
115	0	18	24	42
116	0	8	30	38
117	0	23	20	43

TABLE 2. Case study information for each patient studied at Woodward State Hospital

Index Number	Sex	Date of birth	Parental age at patient's birth			IQ*
			Resident's age	Paternal age	Maternal age	
101	F	11-26-24	49	35-39	30-34	65
102	F	8- 9-46	27	30-34	25-29	Below 30
103	M	1- 7-52	21	35-39	35-39	Below 30
104	F	9-19-42	31	28	25	51-54
105	M	8-26-37	36	30-34	35-39	Below 25
106	M	4-12-50	23	40-49	40-49	Below 25
107	M	6-12-47	26	20-24	20-24	Below 25
108	M	1-18-48	25	25-29	25-29	Below 25
109	M	2- 8-42	31	30-34	25-29	24
110	F	9-29-18	55	30-34	30-34	Below 25
111	F	12-16-51	22	36	39	Below 30
112	M	9- 8-51	22	40-49	35-39	Below 30
113	M	3- 3-53	20	35-39	35-39	Below 30
114	M	4-24-52	21	35-39	35-39	Below 25
115	F	3-10-58	15	25-29	25-29	Below 25
116	M	10- 5-58	15	30-34	30-34	Below 25
117	F	12-19-56	17	40-49	44	Below 25

*Mentality ratings: Mild (IQ 55-69), Moderate (IQ 40-54), Severe (IQ 25-39), Profound (IQ less than 25)

Patient number 101

Patient number 101 is a Down's syndrome female who is short statured, overweight, wears glasses for visual correction and has varicose veins of the lower extremities.

This individual was born prematurely at seven months and delivery was normal. The mother had whooping cough and this was presumed to have induced the premature delivery. The patient is the second of two children.

The patient has a paternal great uncle on the grandfather's side who is a high grade imbecile. This uncle has several children who have been in Glenwood.

Thirty-one cells were counted for this individual with 11 cells showing 46 chromosomes and 20 cells showing 47 chromosomes.

Patient number 102

This patient is a Down's syndrome female who wears glasses for visual correction, has an awkward gait with a built-up shoe on the left foot and varicose veins in the lower legs.

There was no information on the pregnancy history or delivery of this child. The patient is the second of two children.

Of the 41 cells counted in patient 102, 16 showed a count of 46 chromosomes and 25 showed a count of 47 chromosomes.

Patient number 103

This patient is a Down's syndrome male with some visual defects, speech retardation and a slightly awkward gait.

The gestation period was a full nine months and there were no problems in delivery. He is the third of four children.

Cell counts for this patient showed one cell with 45 chromosomes, 12 cells with 46 chromosomes and 23 cells with 47 chromosomes for a total of 36 cells.

Patient number 104

Patient number 104 is a Down's syndrome female, short statured, vision is impaired, head is rather flat in back, no deformity in the body and a systolic heart murmur is present.

This individual was two weeks premature and almost miscarried in early stages of pregnancy. She is the first of four children.

Thirty cells were counted for this individual. Two cells showed a 45 chromosome count, 10 cells showed a 46 chromosome count and 18 cells showed a chromosome count of 47.

Patient number 105

This is a male who presents features typical of an individual with Down's syndrome. The head is about 18 inches in circumference and rounded. The nose is flattened.

The mother did not consult a doctor during the entire pregnancy and the child was born one month premature. He is the fifth of five children born to this woman, the first four children being from a previous marriage.

The maternal grandmother of the father was known to have been feeble-minded. Almost every sibling of the father has a poor education and shows a record of being a slow learner. The first husband had epilepsy and had been previously confined to the state hospital at Cherokee. A sibling of the patient from the mother's first marriage died shortly after birth. One child from the second marriage lived only two days. It was stated by the mother that the child's heart valves did not function properly.

Twenty cells showed a chromosome count of 46 and 10 cells showed a chromosome count of 47. The total number of cells counted was 30.

Patient number 106

This patient is a Down's syndrome male, ambulatory with an awkward gait and no speech.

The gestation period was full term with labor being 24 hours. There were no complications during delivery. He is the sixth child from a family of seven. The other siblings have been normal.

The paternal grandfather died of strep throat at age 62 and the maternal grandmother died of cancer at age 59.

Of the 38 cells counted one showed a chromosome

count of 45, 18 a count of 46 and 19 a count of 47.

Patient number 107

Patient number 107 is a male with typical Down's Syndrome characteristics.

The gestation period was full term with delivery being normal. He is the second of two children.

The patient was a twin and the other twin died a few minutes after birth. There is no record of the twin showing any Down's syndrome characteristics. The patient was spastic at birth and one lung was collapsed.

Cell counts for this individual showed three cells with a chromosome count of 45 and 38 cells with a count of 46 chromosomes for a total of 41 cells.

Patient number 108

Patient number 108 is a Down's syndrome male who is visually handicapped and has a speech defect.

The gestation period was full term. At the time of pregnancy the mother was anemic and had the flu three times during pregnancy. The delivery was in the breech position and the child was reported to be "black at birth". He is the fourth of four children.

A maternal cousin is a patient at Glenwood State School and another maternal cousin is an epileptic at Woodward State Hospital. A third maternal cousin was a patient for three months at the Mental Health Institute in Clarinda, Iowa, but is reported to be recovered from his

mental illness now. A paternal cousin was an individual with Down's syndrome who died at the age of one.

Thirty-four cells were counted. Two showed chromosome counts of 45, 18 showed counts of 46 and 14 showed counts of 47.

Patient number 109

The patient described here is a Down's syndrome male with high cheek bones, ruddy complexion, round head, flattened occiput, epicanthal folds, short stature and shortened long bones with an exceedingly short fifth digit of both hands. The patient is also visually handicapped, hard of hearing and has a speech defect.

The gestation period was full term with no complications during pregnancy. He is the third child of three in the family.

A paternal grandmother has a mentally retarded sister.

One cell showed a chromosome count of 45, 16 cells had a chromosome count of 46 and 13 cells yielded a count of 47. Thirty cells were counted.

Patient number 110

This patient is a Down's syndrome female with a round head and face, epicanthal folds, grey-brown complexion, large wrinkled tongue, short arms and legs, visually handicapped, a speech defect and especially short fifth fingers and toes.

The gestation period was full term with delivery

being normal. She is the fourth of five children.

Of the 42 cells counted, one cell showed a chromosome count of 45, 22 a count of 46 and 19 showed a chromosome count of 47.

Patient number 111

An overweight Down's syndrome female, this patient shows prominent epicanthal folds, rolling of the ears, shortening of the fifth fingers, hypermobility of the joints, separation of the first and second toes, a speech defect and a rounded occipital region.

The gestation period was full term, but the mother had severe neuritis during the last four months. The delivery was in a breech position. She was the third born of three children.

The mother's first pregnancy terminated at three months gestation in a miscarriage.

Thirty-six cells were counted. Eighteen cells showed chromosome counts of 46 and 18 showed counts of 47.

Patient number 112

This patient is a typical Down's syndrome male showing physical underdevelopment, epicanthal folds, wide nasal bones, brachycephaly, broad hands with short fifth fingers, wide feet with wide interval between first and second toes and a large tongue with large papillae.

The gestation period was full term and delivery was normal with the patient being born two minutes after his

twin sister. His twin sister is normal. He is the fourth of six children.

A sister of the paternal grandmother may have been a Down's syndrome individual. The father of the patient believes that she had about the same characteristics as the patient. The mother's cousin had "brain fever" but has completely recovered. Another sister besides the twin sister had cancer of the thyroid gland and has average intelligence. Information regarding other children in the family was not available.

Fifteen of the 35 cells counted showed chromosome counts of 46 and the other 20 cells showed counts of 47.

Patient number 113

Patient number 113 is a Down's syndrome male who presents a protruding tongue, characteristic epicanthal folds, underdeveloped genito-urinary system, flat feet, brachycephaly and short fingers and toes.

The gestation period was full term and delivery was normal. He is the third of four children.

In 1970 the patient was diagnosed as having post-streptococcal acute glomerulonephritis with nephrotic syndrome. He was released from the hospital ward in November of 1971. In October of 1972 he was admitted to the hospital ward with a fever. As of June 1973, he developed chronic nephritis with uremia and hypertension and the prognosis for his recovery was poor.

Forty-eight cells were counted. Nineteen showed chromosome counts of 46 and 29 cells showed chromosome counts of 47.

Patient number 114

This patient is a Down's syndrome male with a very large tongue, clumsy gait, poor speech, flat feet, brachycephaly and strabismus.

The gestation period was eight months and one week and delivery was normal. He is the third of four children.

Of the 40 cells counted 14 showed counts of 46 chromosomes and 26 cells showed counts of 47 chromosomes.

Patient number 115

The patient described here is a Down's syndrome female with encephalopathy due to anoxemia at birth, microcephaly to some extent, a high palate, thick tongue and a chest deformity due to depression of the sternum. The patient is not ambulatory.

The gestation period was full term but delivery was complicated due to the position of the baby. The labor pains were severe lasting for about 10 hours. She is the fourth of four children.

Forty-two cells were counted. Eighteen cells showed chromosome counts of 46 and 24 cells showed chromosome counts of 47.

Patient number 116

Patient number 116 is a Down's syndrome male with a

speech defect, epicanthal folds, shortened extremities especially fifth fingers and toes, nearsighted and an enlarged tongue.

The gestation period was $8\frac{1}{2}$ months long and delivery was normal. He is the oldest of two children.

Eight of the 38 cells counted had chromosome counts of 46 and 30 cells had counts of 47 chromosomes.

Patient number 117

This patient is a female with typical Down's syndrome features.

The delivery was one month early by caesarian section because of the mother's age. The patient is the second of two children.

There was one reported miscarriage which occurred before birth of the patient.

Forty-three cells were counted. Twenty-three of these cells showed counts of 46 chromosomes each and 20 cells showed chromosome counts of 47.

Richards (1969) reported a formula for finding the proportions of mosaic Down's syndrome individuals from normal zygotes and from trisomic zygotes. If the mean maternal age of trisomics is Z , that of mosaics is X and that of controls is N , then P , the proportion of mosaics that started as normal zygotes, equals $(Z-X) / (Z-n)$. This formula assumes that a sample of mosaic individuals with Down's syndrome is a mixture of mosaics of either trisomic

zygotic origin or of normal zygotic origin. Therefore this particular sample of mosaic Down's syndrome individuals will have a mean maternal age at some point intermediate between that of trisomic Down's syndrome and that of normal controls. The degree of reduction of the mean maternal age at birth of mosaics below that of trisomic individuals is a measure of the proportion of mosaics starting from normal zygotes within the mosaic sample.

The results of the present study showed that 59% of the mosaic Down's syndrome individuals were derived from normal zygotes and 41% were derived from trisomic zygotes based on the following calculations.

$$p = \frac{31.5 - 33.8}{31.5 - 27.6} = \frac{2.3}{3.9} = 59\%$$

The maternal ages for the trisomics used for these results were obtained from Pieper (1973) and the control age of 27.6 was taken from Richards (1969).

These results do not agree with some of the other findings of this author in regard to the zygotic origin of the mosaic Down's syndrome individuals in this study, but this could be due in part to the much greater number of individuals used by Richards (1969).

DISCUSSION

Sixteen of the individuals suspected of having mosaic type Down's syndrome by Pieper (1973) were selected and examined in greater detail. Ten other individuals listed as possible mosaics by Pieper (1973) showing one cell with 46 chromosomes and nine with 47 chromosomes were excluded from the study. These 10 were excluded because of the work of Penrose (1961) who excluded alleged mosaics with less than 10% and more than 90% trisomic cells on the grounds that the diagnosis of chromosomal mosaicism, but not of Down's diagnosis, was not convincing. Another individual originally demonstrated to have trisomy 21 by Pieper (1973) was later found to be a mosaic Down's Syndrome individual by Dawson (1974) and is not included in this study.

Based on the 87 Down's syndrome individual's studied by Pieper (1973) at Woodward State Hospital the frequency of mosaic types in this population is 18.4%, of trisomy 21 types is 80.5% and the translocation type individual is 1.15%.

Mikkelsen (1967), Chitham and MacIver (1965), Ziska (1970), Richards et al. (1965), Edgren et al. (1966), Higurashi et al. (1969) and Hayashi (1963) reported a total of 1015 individuals with Down's syndrome in seven separate cytogenetic studies. In the present investigation this total of 1015 individuals was used to calculate the average

frequencies for trisomy 21 individuals, mosaic Down's syndrome individuals and translocation type Down's syndrome individuals. The frequency for trisomy 21 individuals based on a total of 947 patients is 93.3%, for translocation Down's syndrome based on 45 patients is 4.43% and for mosaic Down's syndrome based on 23 patients is 2.26%.

The figure of 2.26% for mosaic Down's syndrome individuals indicates that Woodward State Hospital is not a typical institution with respect to the proportions of mosaic type Down's syndrome. The total number of 87 individual's from Woodward originally studied by Pieper (1973) compares favorably with the 100 Down's syndrome individuals reported by Mikkelsen (1967), the 105 individuals reported by Chitham and MacIver (1965), the 112 individuals reported by Ziska (1970), the 79 individuals reported by Hayashi (1963) and the 73 individuals reported by Edgren et al. (1966) with respect to sample size. These individuals found the percentages of mosaic Down's syndrome to range from 1.78% to 2.85%. Therefore, it would be presumptive to assume that the 18.4% of mosaic Down's syndrome found in the present study was a fortuitous percentage. An increase in the number of Down's syndrome individuals also does not seem to affect the percentage of mosaic Down's syndrome individuals. Richards et al. (1965) reported 2.67% for mosaic Down's syndrome individuals from a total of 225 patients and Higurashi et al. (1969) reported 2.18% for mosaic individuals from a total

of 321 patients. As a result, even though the total population of Down's syndrome individuals at Woodward State Hospital may be somewhat higher than the 87 residents studied by Pieper (1973) it would not be likely that a slight increase in this number would change the 18.4% of mosaic Down's syndrome individuals found in this study to any great extent.

The Down's syndrome individuals at Woodward State Hospital are life long residents who were admitted at an early age, between 1 and 6 months, and the majority have been there since the late 1940's and early 1950's. The ages of the mosaic individuals in this study range from 15 years to 55 years. Patient number 110 in this study was admitted at the age of 53 in 1970. This might indicate that individuals that are not infants but perhaps 6 years or older are now being admitted. With an admissions policy which would not include infants as had been the case previously the mean age would be shifted toward a higher mean age. The mean age found in this study is higher in individuals with mosaic type Down's syndrome than in those individuals with trisomy 21, but this difference does not appear to be significant.

Hayashi (1963) speculated that the severity of expression of Down's syndrome characteristics appears to be related to the proportion of cells with trisomy 21. He also hypothesizes that the fewer the trisomic cells encountered, the greater is the chance that the individual will have

normal intelligence. This however is challenged by other investigators. Kohn et al. (1970), in his study of eight individuals with mosaic Down's syndrome, finds no correlation between the degree of mosaicism and intelligence. He points out that there is a similar over-lapping of the distribution of intelligence between mosaic Down's syndrome and trisomic Down's syndrome. Shipe et al. (1968) points out that there is a great deal of variability in the clinical expression of individuals with nonmosaic Down's syndrome. In some cases the intelligence of trisomic Down's syndrome is as high as 80 (Dunsdon et al., 1960; Zellweger, 1968). Taysi et al. (1970) challenges the attempt to correlate the proportions of trisomic cells in cultured tissues of persons with mosaic Down's syndrome with the phenotypic expression pointing out that the number of trisomic cells is not constant as the individual gets older. Taylor (1970) also showed that rapid cell selection occurs in the small lymphocyte stem cells of young mosaic Down's individuals. She found that the normal cells of seven infants increases with age. Five infants showed an increase of trisomic cells with age and in one boy there was a random fluctuation of chromosome numbers.

Unless previous chromosome counts had been done at an earlier stage in the lives of the 16 mosaic individuals in the present study a comparison could not be made with the counts from this study to see if there was a significant change in the number of trisomic cells present. It would be of interest to compare the mean of chromosome counts in a

follow-up study using new blood cultures from these same 16 individuals to see if there is a noticeable change in the number of trisomic cells present with an increase in age as Taylor (1970) and Taysi et al. (1970) suggest. These counts could also be used to verify chromosome counts from the present study.

In the present study, the average number of cells counted with 47 chromosomes for the 16 suspected mosaics is 20.5 and the average number with a 46 chromosome count is 16.1. Using these figures, based on an average of 36.6 cells counted per individual, the percentage of trisomic cells counted is 56.0% and that of normal cells is 44.0%. This finding is in agreement with the results of Richards (1969) who found a mean of 54.7% trisomic cells and 45.4% normal cells in the population of Down's syndrome that he studied. Richards (1969) also points out that in smaller samples the proportion of trisomic cells in mosaic subjects tends to be less in blood than in skin cultures. He also points out, however, that blood cultures are used by nearly all laboratories as a diagnostic procedure and investigators only resort to supplementary skin cultures if the proportion of trisomic cells in the blood of recognizable mosaic Down's individuals is low. With the percentage of trisomic cells being 56.0% in this study, a follow-up study of fibroblast cultures on these individuals would not be necessary.

Nondisjunction of a trisomic zygote produces triple

stem cell mosaicism whereas anaphase lagging will produce 46/47 mosaicism. Nondisjunction of a normal zygote, provided that it does not occur at the first cleavage, results in 46/47 mosaicism. If it occurs at the first cleavage, a regular trisomic mongol is produced assuming the monosomic 21 is lethal. Anaphase lag of the chromosomes of a normal zygote would not produce a mosaic Down's syndrome individual, but instead will produce a normal cell line and a cell monosomic for chromosome number 21 which tends to die off (Richards, 1969).

When a mosaic arises from nondisjunction of a normal zygote, the later the cleavage of origin, the smaller the proportion of trisomic cells. If nondisjunction arises from a trisomic zygote, the later the cleavage of origin, the larger the proportion of trisomic cells. Also there is a very rapid reduction in the proportions of all cell lines but one, the later the cleavage of origin takes place. For instance, nondisjunction of a normal zygote at first cleavage results in 100% trisomic cells, assuming again that monosomic 21 is lethal. At the third cleavage 33% trisomic cells are produced (Richards, 1969). The range in this study for trisomic cells is from 33% to 79% with a mean of 54.8%. Using this information it would appear that the majority of the 16 mosaics in this study were a result of nondisjunction of trisomic zygotes.

Patient number 104 was almost miscarried early in the

gestation period. Spontaneous abortions are not uncommon among individuals afflicted with chromosomal abnormalities (Larsen and Titus, 1970). Kajii et al. (1973) reported that five of 152 spontaneous abortions were trisomy 21. From the case studies in the present investigation, the mother of patient number 117 had a miscarriage sometime before the birth of this girl, but there is no record of the aborted child's condition and no record of any karyotype being taken.

No significant difference in the prevalence of mosaics among patients born to young or old mothers could be shown by Mikkelsen (1967). Her findings showed two mosaic Down's syndrome individuals from a study of 100 Down's syndrome patients born to young mothers. Richards (1969) found a slight excess of mosaic cases born to young mothers, particularly in the maternal age group 15-19 years. Here the percentages of mosaics born to these mothers was 5.0% whereas the 20-45 age group showed a range from 1.2% to 2.7% incidence of mosaic children. In the present study, the mean maternal age was found to be 33.8 years for the 16 individuals being tested.

Richards (1969) stated that mosaic individuals who start life as normal zygotes should have the same mean maternal age at birth as normal babies, whereas those mosaics that start as trisomic zygotes should have the same mean maternal age at birth as regular trisomic Down's syndrome individuals. The mean age of 33.8 years at the birth

of the mosaics in this study compares closely with the mean maternal age at birth of 31.5 years from the trisomic Down's syndrome individuals studied by Pieper (1973). This evidence seems to be in correlation with the work of Richards (1969).

The incidence of Down's syndrome at birth is about one in 650 and the population incidence of mosaicism in clinically recognizable Down's syndrome is one in 31,000 (Richards, 1969). This assumes no appreciable difference at birth between the mortality of mosaic and non-mosaic Down's syndrome individuals.

Using available life tables and cytogenetic studies on Down's syndrome individuals, an investigation could be conducted to see if the mortality rate of individuals with trisomy 21 is higher than the mortality rate for those individuals with mosaic Down's syndrome. If a significant difference is found in the mortality rates of these two types of Down's syndrome individuals, it could account for the high number of mosaic type Down's syndrome individuals that are present at Woodward State Hospital.

If the literature should produce any similar situations, in regard to the apparent excess of mosaic individuals as there are present at Woodward, comparisons of the institutions could be made using available case histories.

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